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Some observations on an intermediate product in the release of glycopeptide from casein by rennet*)

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Introduction

The unique characteristic of κ-casein, a glycoprotein of milk, had been well recognized through its discrete cleavage by rennet with the release of a glycopeptide containing sialic acid (1—5). κ-casein had been observed to lose virtually all its sialic acid as the glycopeptide (GP) during rennet action (2, 4). The ability of rennet to release the GP from κ-casein is an essential concomitant of function and considered to be the prerequisite for performance of rennet clotting of milk (4).

An evaluation of different milk proteins for their sialic acid levels by GANGULI et al. (6) has recently disclosed that κ-casein and proteose-peptone of milk are the repository of sialic acid in milk. The resemblance in the residence of certain chemical entities like sialic acid and hexose in bound form in κ-casein (6—9), in proteose-peptone (6) and in the GP released from κ-casein (2, 4) are the rationales which has occasioned this study to examine whether proteose-peptone (PP) like component is involved in rennet action.

Materials and Methods

Whole casein and κ-casein were prepared from milk by the isoelectric precipitation (7) and sulfuric acid urea method (10) respectively. Proteose and PP were isolated from milk, deprived of casein and whey-proteins, using ammonium sulphate and trichloroacetic acid (TCA) respectively as the protein precipitants (11). Rennet used was a powder preparation from Hansen laboratory, Copenhagen. Sialic acid (N-acetyl neuramic acid) and thiobarbituric acid were gift samples from Sigma Chemical Company, USA. Glycopeptide was isolated frgm renneted casein by the procedure of GUPTA and GANGULI (3).

Sephadex G-100 was purchased from Pharmacia, Sweden. Starch hydrolysate (Smithies) was purchased from BDH.

Estimation of sialic acid. Sialic acid in casein and glycopeptide was estimated by the thiobarbituric acid assay method as described by GUPTA and GANGULI (7) and in proteose and PP according to GANGULI et al (6). N-acetyl

neuramic acid was used as a standard for expressing sialic acid content in these milk proteins.

Assay system for the release of glycopeptide by rennet: Using as substrate. The release of glycopeptide from casein by rennet was evaluated by the procedure of GUPTA and GANGULI (3).

Using proteose and proteose-peptone as substrate. 20 mg of either proteose or PP was dissolved in 2 ml of N/50 NaOH with gentle heat not exceeding 60 ° C and the pH was finally adjusted to 7.0 with 0.2M phosphate buffer, pH 7.0 and final volume was made to 5 ml. Such solution was then incubated with 0.2 ml of rennet solution (30 mg/ml) and the mixture was incubated for 30 min. at 30 ° C. The reaction was terminated by adding 1.0 ml of 80 % TCA and the precipitated protein was filtered using Whatman No. 42 filter paper. One ml of filtrate was hydrolysed with equal volume of 0.2N H_2SO_4 for 45 min. at 80 ° C. The hydrolysate was cooled and pH adjusted to 4.5 with 0.18 ml of 0.1N NaOH. Finally sialic acid was estimated in this solution using 0.2 ml aliquot. Reaction stopped at zero minute was used as the blank.

Assay system for the estimation of proteose-peptone in renneted milk:

(a) **For whole milk.** Twently ml of milk were incubated with 0.2 ml of a 1.5% rennet solution at room temperature for 15 min. The clotted milk was diluted with 40 ml water and reaction stopped by heating the mixture in a boiling water bath for 15 min. PP was then estimated by the colorimetric method of GANGULI et al. (12). A blank sample was run by incubating 20 ml of milk with boiled rennet.

(b) **For milk deprived of its micellar casein.** Milk was subjected to differential centrifugation at 11,739, 46,956 and 105,651 g (corresponding to 10,000, 20,000 and 30,000 r.p.m.) in a preparative ultracentrifuge for 30 min. (Beckman Spinco, Model L) in order to obtain milk having varying levels of micellar casein. 20 ml of such supernatant preparations were then incubated with 3 mg of rennet at room temperature. The reaction was stopped by heating the mixture in a boiling water bath for 15 min. and analysed for its PP content by the colorimetric method (12). Clotting time for these samples by rennet was recorded by the method of GUPTA and GANGULI (3).

Assay system for rennet action on casein: (a) **For studying the release of PPLM.** 20 ml of 1% casein in N/50 NaOH adjusted to pH 7.0 with phosphate buffer were incubated with different concentrations of rennet solution (from 10 to 100 mg per assay system) at 37 ° C for 30 min. The reaction was terminated by precipitating the casein at pH 4.6 with acid. The filtrate thus obtained was analysed for PPLM and non-protein fractions (TCA-soluble components) by the colorimetric procedure of GANGULI

et al. (12). A blank was similarly run without rennet. Experiments were also carried out at different periods of incubation with 100 mg rennet in the assay system.

(b) **Estimation of total sialic acid and sialic acid of PPLM and GP released from casein by rennet.** 100 mg of casein in 2 ml of N/50 NaOH adjusted to pH 7.0 with 3 ml of phosphate buffer was incubated with 0.1 ml rennet (30 mg/ml) for 20 min. at 30 ° C. The reaction was stopped by the isoelectric precipitation of casein through pH adjustment to 4.6. The supernatant containing the released intermediate and GP was analysed for their sialic acid content. Total sialic acid in the supernatant was determined after acid hydrolysis according to GUPTA and GANGULI (3, 7). PPLM was then precipitated from the casein-free supernatant by TCA (11) and sialic acid again estimated in the casein-PPLM-free filtrate which was recorded as glycopeptide sialic acid. Sialic acid in PPLM was evaluated by subtracting the GP sialic acid value from the total sialic acid.

(c) **For isolation of the intermediate product (PPLM) from the reaction mixture.** 20 ml of 1 % casein (prepared as indicated above) was incubated with 100 mg rennet at 37 ° C for 15 min. The reaction was stopped by isoelectric precipitation of casein and five such incubated samples were mixed together for the subsequent isolation of PPLM from the casein-free filtrate using TCA by the method of GANGULI et al. (11). Control samples were as well analysed which were incubated with boiled rennet.

(d) **Estimation of sialic acid in the released PPLM.** Sialic acid content of TCA and ammonium sulphate insoluble components isolated from casein-rennet mixture was determined by hydrolysing the sample with 1N H_2SO_4 for 45 min. at 80 ° C (7). The hydrolysate was further processed according to the method of GANGULI et al. (6, 7) for the estimation of sialic acid.

Gel filtration of proteose-peptone from milk on Sephadex G-100: (a) **Preparation of gel column.** Gel filtration was carried out on Sephadex G-100 on column of 2.5 cm × 40 cm. The gel was normally allowed to soak in distilled water for 48 hours at room temperature before use. Before running the protein sample, the gel column was equilibrated with the eluting buffer which was Tris-HCl, 0.01M of pH 8.0 containing 0.3M NaCl.

(b) **Preparation of the sample for gel filtration.** 16 - 20 mg of either proteose or PP sample isolated from milk was dissolved in 2 ml of same eluting buffer. The whole sample was then placed gently on the top of the column bed.

(c) **Gel filtration procedure.** Gel filtration was followed according to the published literature (13) and fractions collected using the above buffer as the eluting solvent in an automatic fraction collector. 0.5 ml aliquot of eluted

buffer was analysed from each tube (containing 6 ml) for its protein estimation by the method of LOWRY et al. (14). The developed color was read in Klett Summerson photoelectric colorimeter using red filter. The results were finally expressed graphically.

Determination of sedimentation coefficient and molecular weight of proteose and proteose-peptone from milk. Sedimentation velocity experiments for proteose and PP were conducted in an analytical ultracentrifuge (Beckman Spinco, Model E) employing analytical accessories and a rotor speed of 59,780 r.p.m. The protein concentration used was 1% in veronal buffer of pH 8.6 and 0.1 ionic strength. Molecular weight was determined by the ARCHIBALD method (15). For the calculation of the molecular weight a partial specific volume of 0.75 has been assumed.

Starch gel electrophoresis. Electrophoretic resolution of the protein fraction was carried out by the gel electrophoretic method of El-Negoumy (16).

Separation of peptides. The renneted whey obtained from casein was dried **in vacuo** at room temperature and residue dissolved in water (usually in 1—2 ml). This extract was subjected to finger printing using the procedure of GANGULI et al. (17) and peptides detected by spraying the chromatogram with 0.2% ninhydrin in acetone.

Results

The release of PPLM in milk on rennet action. Since rennet releases a GP containing sialic acid from casein and PP a sialic acid containing component in milk, it was thought worthwhile to examine the fate of PP and its level in milk after rennet action on milk. Two similar aliquots of the same milk sample were analysed to evaluate the content of PP using active rennet. Results in Table 1 indicate that milk samples after rennet treatment gave a distinct increase in the PPLM compared to the original concentration of PP in milk. Since the samples treated with boiled rennet did not exhibit any change in PP level an increase in the milk treated with rennet can then be attributed to the enzymatic release of PPLM from milk proteins. Both cow and buffalo milk responded in a similar manner in this respect.

Proteose-peptone content of milk with different levels of micellar casein before and after rennet action. In order to characterize the fraction in milk responsible for the increased PPLM in milk as a result of

rennet action, the subsequent studies were centered around casein, the major sialic acid positive rennet substrate, present in milk. Experiments were first designed to deprive milk of its micellar casein by differential ultracentrifugation and then the effect of rennet on such milk preparations was studied in relation to proteose-peptone release during rennet action. PP was estimated in these milk samples before and after rennet action. It is apparent from the results in Fig. 1 that with the removal of micellar casein from milk, the release of PP in milk decreased with an increase in milk clotting time during rennet action. Furthermore, the release of PP was more in all cases with cow milk than buffalo milk.

Table 1. Proteose-peptone content of milk before and after rennet action

Nature of milk	No. of experiments	Original milk		Milk+active rennet		Milk+boiled rennet	
		Range	Average	Range	Average	Range	Average
Cow	5	185—640	278	430—613	530	200—325	255
Buffalo	5	150—320	225	340—565	475	135—300	287

The release of glycopeptide from casein, proteose and proteose-peptone of milk by rennet. The release of glycopeptide (8 % TCA soluble bound sialic acid components) from casein, proteose and PP by rennet was first evaluated in order to establish whether rennet is capable of utilizing these sialic acid positive fractions of milk like casein. It appears from results in Table 2 that rennet is capable of releasing the sialic acid present in either proteose or PP in bound form like its release from casein. In cases of proteose and PP from buffalo milk, the release appears to be more complete than in cow samples whereas reverse is the case with casein samples from these species of animals.

Release of PPLM from casein by rennet: (a) With rennet concentration. After observing a probable correlation between the casein content of milk and PP released by rennet in milk, an attempt was made to

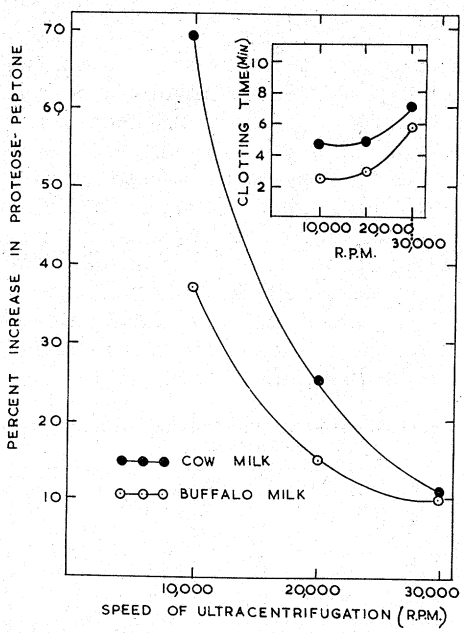

Figure 1. Rate of release of proteose-peptone from milk by rennet with the removal of micellar casein.

detect the release of PPLM when rennet acts on casein alone. Fig. 2 depicts the results on the relative release of PPLM and non-protein fractions from casein with increase in rennet concentration. These values were always corrected for blank values with boiled rennet. Results indicate that rennet does release PPLM from casein and there exists a proportional increase in such release with rennet concentration in the assay system. There is also a simultaneous release of non-protein fraction along with PPLM. With increase in enzyme concentration, the ratio of PPLM to non-protein gradually decreased. It has been further observed that calcium ion does not influence the release of PPLM from casein during rennet action.

Relative release of PPLM and the glycopeptide from casein by rennet. It appears from above data that rennet is capable of releasing both PPLM and GP

Table 2. Release of sialic acid as glycopeptide from milk proteins by rennet

Animal	Substrate	Initial sialic acid mg/g.	Bound sialic acid released (%)	
			Before rennet action	After rennet action
Cow	Casein	5.7*	0.50	90.0*
	Proteose-peptone	25.4**	0.20	94.4
	Proteose	32.6**	0.12	89.2
Buffalo	Casein	2.5*	0.30	60.0*
	Proteose-peptone	9.6**	0.18	100.0
	Proteose	11.5*	0.22	100.0

* Data from Gupta and Ganguli (3)
** Data from Ganguli et al. (6)

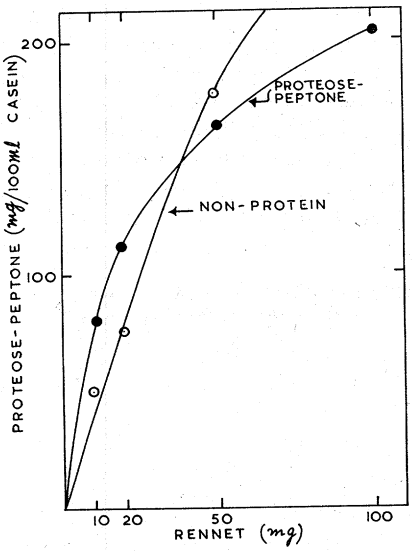

Figure 2. Release of proteose-peptone and non-protein fractions from milk with increasing concentration of rennet.

from casein. The relative proportions of these two released products was then evaluated. The release of these components is quite evident from the results in

Table 3. Under the conditions of experiments, there appears to be a greater release of PPLM than GP from cow casein whereas reverse was the case with buffalo casein.

Isolation and characterization of PPLM released from casein by rennet. After observing a definite release of PPLM from casein due to rennet action, the next essential step would be to study the physico-chemical characterization of the released intermediate. Mainly three criteria were adopted for such purpose: (a) its electrophoretic pattern, (b) its sialic acid content and (c) its gel filtration pattern on Sephadex. Large scale incubations using 100 mg rennet per 20 ml of 1 % casein were carried out and PPLM from every incubated mixture was isolated by the published procedure (18) and subjected to analysis.

a) **Electrophoretic pattern of the PPLM and PP from milk.**

For a better appraisal of samples of PPLM isolated from casein and PP from milk were examined by starch gel electrophoresis (16). It has been observed that the isolated PPLM from casein digested with rennet exhibited similar electrophoretic behaviour as that of native PP of milk. These samples resolved into two major components with similar mobilities in both cases.

Table 3. Relative release of proteose-peptone and glycopeptide from casein by rennet

Components analysed	Sialic acid (mg/g)		Present of total sialic acid released	
	Cow	Buffalo	Cow	Buffalo
Total sialic acid released	2.783	1.521		
Proteose-peptone sialic acid	1.753	0.634	62.9	41.7
Glycopeptide sialic acid	1.035	0.887	37.10	58.3

b) **Sialic acid content of PPLM isolated from renneted casein.** Since sialic acid is a typical sugar moiety present in PP, an evaluation of the same in the isolated PPLM from casein was expected to elicit useful information as regards its chemical make up. The rele-

ased PPLM was observed to resemble the native PP in milk in its sialic acid content. Proteose-like samples isolated from renneted casein using ammonium sulphate (11) have been observed also to exhibit similarity in its physico-chemical properties with native proteose isolated from milk.

c) **Gel filtration pattern of PPLM isolated from renneted casein.** A detailed study on the gel filtration pattern of PP isolated from milk on different grades of Sephadex by GANGULI et al (19) has revealed the presence of two components in PP having different filtration rates. The faster moving one was identified to be proteose whereas the slow moving component

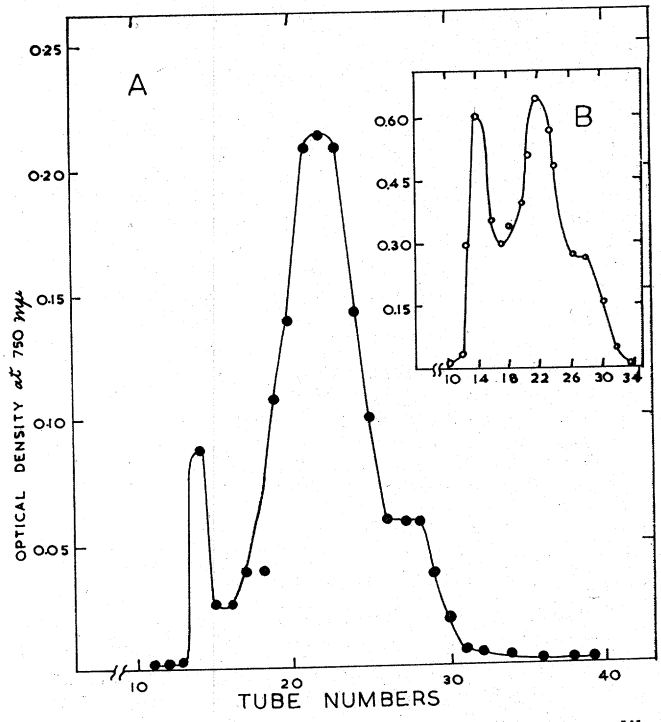

Figure 3. Gel filtration pattern of proteose-peptone like material (PPLM) released from $\varkappa$-casein by rennet. A — PPLM from renneted $\varkappa$-casein; B — Native proteose-peptone from milk.

Table 4. Molecular weight data on proteose and proteose-peptone of milk

Sample No.	Nature of sample	Rotor speed r. p. m.	Duration of run (min)	Molecular weight
B27P	Proteose	16,200	15	17,550
			30	16,140
			45	14,370
B27PP	Proteose-peptone	15,220	15	11,050
			30	10,000
			45	10,020

Table 5. Overall comparison between casein, proteose, proteose-peptone and glycopeptide

Physico-chemical properties	Casein	Proteose-peptone	Proteose	Glyco-peptide
Sialic acid (%)	0.57[a]	2.54[a]	3.26[a]	11.3[d]
Hexose (%)	1.90[d]	2.30[b]	2.78[b]	7.4[d]
$S_{20}w$	1.18[c]	2.3[a]	1.6[a]	1.5[d]
Molecular weight	53,600[c]	10,390[a]	16,000[a]	8,000[d]
Isoelectric point	4.6[c]	—	4.5—6.0[f]	2.8[d]
Solubility in TCA	Insoluble	Insoluble	Insoluble	Soluble
Amino acid number	19[f]	17[f]	17[f]	11[e]
N-terminal amino acid	Argi-nine,[g] Lysine	Serine[f] Cystine	Serine[f] Cystine	None[d]

a — Data from the present study;
b — Data from Ganguli et al. (6);
c — Data from Thompson et al. (28);
d — Data from Lindqvist (4);
e — Data from Nitschmann and Beeby (5);
f — Data from Ganguli et al. (11);
g — Data from Ganguli et al. (29).

was peptone. Similarly, the PP from milk and sample isolated from renneted casein, PPLM, were subjected to gel filtration on Sephadex G-75 to study their relative pattern and final characterization. It appears from the pattern depicted in Fig. 3 that there are two fractions in PPLM resembling the protein peaks due to proteose and peptone.

Ultracentrifugal pattern, sedimentation coefficient and molecular weight of proteose-peptone and proteose

isolated from milk. Since PPLM has several similarity in its physico-chemical behaviour with that of PP from milk, the ultracentrifugal pattern and molecular size of PP was therefore compared with GP values (4). One typical sample of proteose (B27P) and of PP (B27PP) isolated from the same buffalo milk were subjected to such analyses. Tables 4 and 5 show these results. The sedimentation velocity patterns are depicted in Fig. 4, upper pattern for proteose and the lower one for PP. The ultracentrifugal patterns of proteose appear to resemble the published data on the pattern of GP in its number of peaks and S_{20}, w values (2). The molecular weight of proteose (16,000) was as well found to be the double of that of the GP (8 000) (8).

The release of peptides from casein during rennet action. It has been observed that during rennet action on casein there was an increase in non-protein (NP) fractions (Fig. 2) which apparently comprise the GP and the soluble peptides, if any. The presence of several peptides (about 10) was observed in the TCA soluble fraction when subjected to finger printing (17), some of which were charged (either positively or negatively) and some were neutral at the particular pH used (6.5) for their electrophoretic separation.

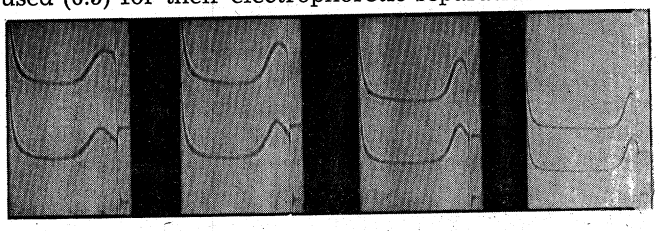

Figure 4. Ultracentrifugal patterns of proteose (upper) and proteose-peptone (lower) isolated from milk.

Comparative appraisal of the physico-chemical properties of casein, PPLM, PP and GP. For assessing the overall outcome of the present study, it is felt essential to compute the physico-chemical data of casein, PPLM, PP and GP with a hope to correlate the sequence of reactions involved in the release of these fractions from casein by rennet. To meet such purpose, the analytical values of sialic acid, hexose, total car-

bohydrate, sedimentation coefficient, molecular weight, TCA solubility and other analytical data for the fractions were compiled in Tab. 5. It is quite evident from the physico-chemical data that these fractions have similarity in many aspects in their chemical composition such as they are all glycoproteins containing sialic acid and hexose. On the basis of the relative increase in these components and subsequential decrease in molecular weights and amino acid number, these fractions can be placed in the following sequence: Casein, proteose, PPLM and GP.

Discussion

The first constructive evidence to suspect a correlation between casein, PPLM, PP and GP in relation to rennet action is the presence of sialic acid in these fractions having different levels of concentration (Table 1). The highest sialic acid content in the GP (2), 113.0 mg/g, as against 32.6 mg/g for proteose and 5.6 mg/g for casein suggests the probable removal of the non-glycoprotein part from casein and proteose by rennet and thereby ultimately result in a maximum concentration of sialic acid in GP per unit weight. Secondly, susceptibility of both PP and proteose native in milk like casein towards rennet (Table 1) in relation to the GP release from these fractions immediately elicits these milk components to be additional substrates for rennet. Similar observations on PP as a rennet substrate was reported by Brunner and Thompson (20) earlier.

The next observations on the increase in PPLM in milk incubated with rennet (Table 1) and its subsequent decrease with the removal of micellar casein from milk (Fig. 1) again indicate casein to be responsible substrate for the appearance of PPLM as its cleaved product by rennet. These observations may probably be explained in the light of the following reaction steps:

(i) Casein → Para-casein + PPLM (Reaction a)
(ii) PPLM → GP + Peptide (Reaction b)

Through „Reaction a" it might be possible to explain an increase in PPLM (Table 1) in milk. „Reac-

tion b" as well had been demonstrated to take place through rennet action (Table 2). A logical explanation of these changes caused by rennet in milk would be to release PPLM released from casein with rennet concentration (Fig. 2) satisfies the typical enzyme kinetics. The simultaneous increase of non-protein components (Fig. 2) is not surprising as the release of soluble substances others than GP during rennet action on casein was also observed by Alais et al (1) which apparently contributes to the non-protein fraction. PPLM released from casein by rennet remains unaltered on the addition of calcium in the incubation system. This is not surprising since calcium helps in the non-enzymatic step in clot formation (4) and not in the renneted cleavage of casein.

A steady increase in milk clotting time was observed with the removal of micellar casein from milk (Fig. 1) which is quite expected since casein is the rennet substrate. Whereas the release of PPLM from milk by rennet had decreased with the removal of micellar casein from it. These findings further suggest casein to be the substrate for the release of the intermediate product (PPLM) by rennet. The lesser release of PPLM from buffalo milk by rennet can probably be attributed to the lower proportion of α-casein in buffalo milk casein than cow milk casein (21). Since α-casein is the substrate for rennet action, one would expect a relatively lower value of this component in buffalo than cow milk.

It is quite evident from the results on the release of PPLM and the GP from casein by rennet (Table 3) that both fractions were released and of the total sialic acid in casein almost 63% appeared as PPLM sialic acid. Cow and buffalo casein, however, showed different patterns in the release of these fractions.

The data on the characaterization of the intermediate product (Fig. 3) provide reasonable support to identify the released fraction resembling PP type of an intermediate. The electrophoretic property and sialic acid content of this intermediate also resemble such values of PP isolated from milk (6,11).

From the ultracentrifugal studies on PP and proteose (Tables 4,5 and Fig. 4) of milk, it appears that these results concur with the data for PP reported by BRUNNER and THOMPSON (20). The pattern of proteose resembles the sedimentation pattern of GP as reported by others (2). These data also indicate that the molecular weight of GP, 8,000, as reported by others (8) appears to be half of that of proteose, 16,000 (Table 4), a fraction which resembles PPLM.

There exist as well previous reports (22, 23) proposing proteose as a released product from casein by rennet. KIRCHMEIER reported (24) that acid serum contained two proteose components, rennin serum contained three of which two were identical with those in the acid serum and the third being released due to rennin action. NITSCHMANN and HENZI (25) isolated nine peptides liberated from whole casein by rennet. It is also evident from our results that peptides are released during rennet action on casein and such action is normally attributed to the tertiary action of rennet (4). Hence it is tempting to postulate the appearance of a proteose type of intermediate first from casein and subsequent proteolysis of proteose might reasonably give rise to the GP and peptides and such phenomenon will be quite compatable with the demonstrated results indicated above. Such a contention gains further support from the remarks made by BEEBY (26, 27) that the GP is formed when a fraction that is first released from the $\varkappa$-casein is subsequently split by the enzyme. From the report of BEEBY and NITSCHMANN (27) it is also apparent that the initial action of rennin involves the dissociation of the $\varkappa$-casein complex into monomers by opening the secondary bonds. The molecular weight of the monomer is 16,000 which might then be resembling the intermediate proposed in this study as a component like proteose.

Although the reactions studied on casein and results presented in this paper are not with a crystalline rennin preparation, it is, however, not possible to rule out the present findings with crude rennet on the basis of enzyme purity. Observations with crystalline rennin are expected to fortify these results (26, 27).

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Zusammenfassung

Ganguli, N. C., Joshi, V. K., Gupta, S. K., and Bhalerao, V. R.: **Einige Beobachtungen über ein Zwischenprodukt aus der Abspaltung von Glycopeptid aus Casein durch Labeinwirkung.** *„Milchwissenschaft“* **23.** *(6) 334—339 (1968).*

24 Casein (Labeinwirkung), **Lab** (Caseinspaltung).

Die Milch scheint durch die Einwirkung von Lab ihre Proteose-Pepton-Fraktion anzureichern, wie es sich durch eine Erhöhung des trichloressigsäure-unlöslichen Materials im Casein und im molkenproteinfreien Filtrat gezeigt hat. Milch, der das mizelläre Casein entzogen worden war, zeigte in solcher Fraktion nur sehr geringe Änderung durch Labeinwirkung.

Die Abgabe eines proteose-pepton-ähnlichen Materials (PPLM), das in Trichloressigsäure unlöslich ist und eines Glycopeptids aus Casein durch Lab hat gezeigt, daß die Anteile dieser Fraktionen in Kuh- und Büffelmilch-Caseinen unterschiedlich sind. Das durch Labeinwirkung von Casein abgegebenen PPLM hat (a) ähnliches elektrophoretisches Verhalten, (b) ähnliche Neuraminsäurereste und (c) ähnliches Gelfiltrationsbild wie das Proteose-Pepton von Milch.

Auf der Grundlage der physikalisch-chemischen Eigenschaften des PPLM wird die Abgabe eines proteoseähnlichen Zwischenproduktes vor der Spaltung durch Lab in Glycopeptide aus dem $\varkappa$-Casein vermutet. Dok.-Ref.

Ganguli, N. C., Joshi, V. K., Gupta, S. K., and Bhalerao, V. R.: **Some observations on an intermediate product in the release of glycopeptide from casein by rennet.** *„Milchwissenschaft“* **23.** *(6) 334—339 (1968).*

24 Casein (rennet action), **rennet** (casein cleavage).

Milk on rennet action appeared to enrich in its proteose-peptone fraction as revealed by an increase in the trichloroacetic acid-insoluble material in casein and whey proteins free filtrate. Milk deprived of its micellar casein exhibited very little change in such fraction on rennet action.

The release of a proteose-peptone-like material (PPLM), insoluble in trichloroacetic acid and glycopeptide from casein by rennet had been demonstrated, proportions of these fractions being different in case of cow and buffalo milk caseins. The released PPLM from casein by rennet have similar (a) electrophoretic behaviour, (b) sialic acid residue and (c) gel filtration pattern like proteose-peptone of milk.

On the basic of the physico-chemical properties of the PPLM, the release of a probable intermediate like proteose before its cleavage to glycopeptide from $\varkappa$-casein by rennet has been suspected.

GANGULI, N. C., JOSHI, V. K., GUPTA, S. K., et BHALERAO, V. R.: **Quelques observations sur un produit intermédiaire provenant du clivage de la caséine en glycopeptide par l'action de la présure.** *„Milchwissenschaft"* **23.** *(6) 334—339 (1968).*

24 **Caséine** (action par la présure), **présure** (clivage de la caséine).

GANGULI, N. C., JOSHI, V. K., GUPTA, S. K., y BHALERAO, V. R.: **Algunas observaciones sobre un producto intermediarie proveniente del desdoblamiento de la caseína en glicopeptidos por la acción del cuajo.** *„Milchwissenschaft"* **23.** *(6) 334—339 (1968).*

24 **Caseína** (acción del cuajo), **cuajo** (disociacción de la caseína).